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PHARMACOLOGY AND METABOLISM OF COMPOUND A

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Food and Drug Research Laboratories, Inc.

DECEMBER 1967

AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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FOREWORD

This study was sponsored by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio 45433. The research was performed in accordance with Contract No. AF33(615)-2380 and Modification No. 1 thereof and in support of Project 6302, "Toxic Hazards of Propellants and Materials," and Task 630202, "Pharmacology-Biochemistry." Dr. Myron S. Weinberg was principal investigator and Richard E. Goldhamer was co-investigator for the Food and Drug Research Laboratories, Inc., Maurice Avenue at 58th Street, Maspeth, N. Y. 11378, and Dr. Kenneth C. Back was the contract monitor for the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory, Aerospace Medical Research Laboratories. Research was initiated on 1 March 1965 and completed on 31 August 1966.

Publication of this report does not constitute Air Force approval of the report's findings or conclusions. It is published only for the exchange and stimulation of ideas.

Wayne H. McCandless
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ABSTRACT

Studies are described in which parenteral, oral, topical, and inhalation administration of Compound A (ClF_5) have been made to rats, cats, guinea pigs, and rabbits. Administration of microliter quantities of the material caused traumatic explosions and death in most animals. The notable findings in survivors reflected protein alterations which were considered to be sequelae to massive evolution of the energy of hydrolysis and/or decomposition. No pharmacological or biochemical activity of the material could be demonstrated at tolerated doses.

SECTION I

INTRODUCTION

Studies were carried out to determine the pharmacological activity and the metabolic fate of Compound A, (ClF_5). Although the results of inhalation exposures of congeners have been reported, there are no summaries of effects of parenteral, oral, or dermal exposures to these materials, or to the biochemical or metabolic sequelae to administration by any route. The project was planned to determine pharmacological effects by evaluation of behavioral, physical, and biochemical changes induced by administration of the material. Preliminary studies were carried out to establish maximum tolerated doses. For this purpose it was elected to follow alterations in hepatic, renal, and metabolic function at those tolerated levels. The procedures employed included tests of dye excretion, blood coagulation, enzyme levels (serum and tissue transaminases as indices of cellular metabolism), blood chemical levels (urea nitrogen, glucose), and nitrogen balance studies to permit evaluation of the general physiological status of the test animals as affected by the exposure.

The initial problems which required solutions prior to start of these studies involved the development of suitable, safe techniques for handling and administration of the test material. Following this preliminary phase, biochemical studies were undertaken to determine applicability of the methods suggested for use in following the metabolic fate of Compound A.

SECTION II

MATERIALS AND METHODS

ADMINISTRATION OF TEST MATERIAL

The method of administration by inhalation followed the general procedure described by Dost et al (ref 1). The design of the basic equipment was modified slightly, but the chamber used conformed essentially to their design. Laboratory personnel working with this material were equipped with masks fitted to compressed air lines, but no specific alterations were made in the room in which the work was carried out. The entire room was vented to the outside using an ultrafiltration system designed by these Laboratories in conjunction with the Army Chemical Corps and the Mine Safety Appliance Corporation to prevent contamination of the surrounding atmosphere.

Parenteral, oral, or topical administration was made using Hamilton Microliter Syringes which were prerinsed with KF-10* to prevent their destruction. Aliquots of 0.5 ml of the liquid were decanted into platinum crucibles which were maintained in a dry helium atmosphere. Material was withdrawn into the syringe and applied or administered as required. At the end of an experiment, the remainder in the crucible, usually 0.2 ml or less, was destroyed in an explosion pit. At this level of exposure, the risk of explosion was minimized and there was little hazard to the technicians.

Fluoride Analysis

The metabolism of Compound A was followed by determination of blood and tissue fluoride levels (ref 2). Preliminary results indicated that the limit of sensitivity, using the procedure of Hall and Weinstein, under our conditions was 20 mcg per ml of blood or tissue. The values reported thus indicate that fluoride content did not exceed these concentrations. No emphasis was placed on the normal levels, toxicological ramifications being associated with frank and significant elevations in fluoride content. In the publication AMRL-TR-65-223 parenchymal tissue and liver were reported to contain 10 to 14 mcg of fluoride per g wet weight and the lung 5 to 7 mcg per g wet weight. Thus, in the following section where fluoride levels are reported as 0, fluoride, if present, was below 20 mcg per g following exposure to Compound A.

*KF-10, Kel-F is available from Minnesota Mining and Manufacturing Company of St. Paul Minnesota.

SECTION III

PROCEDURES

The summary of all intravenous, intraperitoneal, intragastric, subcutaneous, dermal, or inhalation administration is shown in tables Ia, b, c, and d and includes a listing of the biochemical and other studies carried out.

Blood fluoride analyses were made by the modified method of Hall and We instein which was described by Reed et al (ref 2). For the determination of serum enzymes, the methods recommended by the Sigma Chemical Company of St. Louis, Missouri (ref 3) were used. Tissue enzyme analyses were carried out by the methods of Sumner and Myrback (ref 4). Blood coagulation was measured using procedures described by Biggs and McFarlane (ref 5) viz, the one-stage prothrombin time method of Quick, the capillary tube coagulation time technique, the Lee-White clotting time technique, the thromboplastin generation test of Hicks and Pitney, and the partial thromboplastin time test of Biggs and Douglas. Indocyanine green retention was determined by the method of Ketterer et al (ref 6). Plasma protein and hemoglobin electrophoresis, blood urea nitrogen and glucose levels, tissue glycogen levels, and nitrogen balance studies were determined by the methods described by Oser (ref 7).

TABLE Ia

SUMMARY OF PROTOCOL IN RATS

Route of Administration	No. per Group	Dose per Animal	Determination
		µl	
Subcutaneous	3	10	Mortality
	3	25	"
	3	50	"
Intraperitoneal	3	10	"
	3	25	"
	3	50	"
Intravenous	3	10	"
	3	25	"
	3	50	"
Intragastric	3	10	"
	3	25	"
	3	50	"
		ppm	
Inhalation	6	400, in air for 60 min	"
	6	400, in air for 5 min	"
	16	400, in air for 10 min	Sacrificed immediately after exposure; respiratory enzyme activity in the lungs. Groups of 3 rats sacrificed immediately, 16, and 24 hours after exposure. Respiratory enzymes, indocyanine green excretion, glucose, or glycogen, and electropherograms of protein in blood and lungs.
	40	100, 15 min per day	Food intake and urine nitrogen output each day; body weights initially and at termination. Groups of 3 rats sacrificed immediately and 16 hours after each exposure. Enzyme activity and fluoride determinations in lung, liver, and serum; indocyanine green excretion; serum and hemoglobin electrophoresis, and hexobarbital sleeping time.
	15	400, 10 min	Clotting time, coagulation time, prothrombin time, thromboplastin generation time, partial prothromboplastin time, and fibrinogen level in cardiac blood immediately postexposure.
	24	100, 15 min per day, 6 days	Fluoride and prothrombin time in blood; respiratory enzyme activity in liver and lung tissues in samples taken from 3 rats after each exposure and 24 and 48 hours after test exposure.

TABLE Ib

SUMMARY OF PROTOCOL IN CATS

Route of Administration	No. per Group	Doses per Animal	Determinations
		µl	
Subcutaneous	2	10	Mortality
	2	25	"
	2	50	"
Intraperitoneal	2	10	"
	2	25	"
	2	50	"
Intravenous	2	10	"
	2	25	"
	2	50	"
Intragastric	2	10	"
	2	25	"
	2	50	"

TABLE Ic

SUMMARY OF PROTOCOL IN GUINEA PIGS

Route of Administration	No. per Group	Dose per Animal	Determinations
		µl	
Subcutaneous	2	10	Mortality
	2	25	"
	2	50	"
Intraperitoneal	2	10	"
	2	25	"
	2	50	"
Intravenous	2	10	"
	2	25	"
	2	50	"
Intragastric	2	10	"
	2	25	"
	2	50	"

TABLE I d

SUMMARY OF PROTOCOL IN RABBITS

Route of Administration	No. per Group	Dose per Animal	Determinations
		µl	
Subcutaneous	2	10	Mortality
	2	25	"
	2	50	"
Intraperitoneal	2	10	"
	2	25	"
	2	50	"
Intragastric	2	10	"
	2	25	"
	2	50	"
Intravenous	3	100, injected slowly over 15 min	Mortality. Blood fluoride levels; hemoglobin electrophoresis, and brain, heart, liver, and lung GOT
	2	10	Survival
	2	25	"
	2	50	"
	3	20, by slow infusion for 15 min	SGPT, SGOT, serum protein and hemoglobin electrophoresis, blood fluoride, BUN, and glucose 0, 15, 30, 60, and 360 minutes after administration.
	4	10, by slow infusion for 15 min	As above
	3	1	As above
	3	1 per day for 5 days	Prior to the first and 5th injection, blood fluoride, GOT, and prothrombin time. Necropsy after 5th injection.
	3	1 per day for 20 consecutive days	Prior to 0, 5th, 10th, and 20th injections, blood fluoride, GOT, and prothrombin time. Necropsy after 20th injection.
Dermal	1	10	Skin section examined for fluoride, GPT, and GOT, serum fluoride, glucose, urea nitrogen, GPT, and GOT.

SECTION IV

RESULTS

RAPID ACUTE ADMINISTRATION

Acute intraperitoneal, intravenous, or intragastric administration of 10, 25, or 50 µl of Compound A to rats, rabbits, guinea pigs, and cats by rapid (less than 1 minute) administration resulted in the immediate death of the animal. Some convulsions immediately prior to death were noted among animals receiving intravenous doses. Most frequently, this route caused destructive rupture of the blood vessels with hemorrhage of the adjacent area. Subcutaneous administration resulted in traumatic rupture of the skin surrounding the site of injection with massive blood loss followed by respiratory depression, cardiac failure and death. Signs of shock were seen in both groups of animals and death was due to localized destruction of the vascular beds. Necropsy of animals treated by intraperitoneal injection or intragastric intubation revealed massive accumulations of unclotted blood throughout the entire abdominal and thoracic cavities, raising questions of effects of Compound A on coagulation. Shock appeared to be the cause of death.

No other studies were carried out using this technique of rapid parenteral or oral administration of the test compound.

A single rabbit was treated topically with 10 µl of the compound applied to the depilated intact skin in the dorsal area. Signs of dermal corrosion with marked peripheral vasodilatation were noted. Evidence of pain in the treated area was noted. The results of biochemical examination of skin and blood are shown in table II with data from a control rabbit for comparison, showing depression of skin enzymic activity. For humane reasons the rabbit was sacrificed within 10 minutes of treatment.

TABLE II

FINDINGS IN ONE RABBIT RECEIVING 10 μl
BY DERMAL ADMINISTRATION

	Control*	Treatment
Skin Fluoride, μg per g	0	0
Glutamic Oxaloacetic Transaminase		
serum, units per ml	21	36
skin, units per 100 g	1500	0
Glutamic Pyruvic Transaminase		
serum, units per 100 ml	20	16
skin, units per 100 g	1621	0
Blood Glucose, mg per 100 ml	48	61
Blood Urea Nitrogen, mg per 100 ml	13.0	14.6

*Selected at random from the stock colony maintained by these Laboratories.

Inhalation Studies

Exposure of rats to air containing 400 ppm Compound A for 60 minutes resulted in the death of all animals early in the course of the exposure. Postmortem studies were not carried out.

Three rats died during the 10-minute exposure to 400 ppm in air, while the remaining three died within 15 minutes thereafter.

Of the third group of six rats, three that were exposed to 400 ppm in air for 10 minutes survived and were sacrificed immediately after exposure. The gross findings at necropsy included marked pulmonary edema and hemorrhage, myocardial infarction, and congestion in the liver and brain. No respiratory enzyme activity, i.e., glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), ornithine carbamyl transferase, lactic dehydrogenase, or isocitric dehydrogenase could be detected in the lung tissues collected from these three rats.

Of the 10 rats exposed to 200 ppm in air for 10 minutes, 9 survived exposure. The findings in the groups of 3 rats sacrificed immediately, 16, or 25 hours postexposure, are given in table III. In general, abnormal proteins (macroglobulins between the β and γ fractions) were noted in the pulmonary tissue extracts at all periods indicating some denaturant effect following inhalation exposure to 200 ppm of the test material.

No respiratory enzyme activity was found in the lung (alveolar) tissue at the conclusion of the exposure although such activity was seen 16 and 24 hours after exposure. This finding indicates alveolar destruction. However, it was evidently limited in degree (reversibility) since tissue enzymes were either regenerated, synthesized, or made available during the first 16 postexposure hours. No systemic effects were noted, as determined by indocyanine green retention or blood glucose levels. When one considers that the tidal volume of the rat averaged 1.3 ml and the respiratory rate 100 ml per minute, the maximum total inhalation during a 10-minute exposure to 200 ppm by an average rat would be a dose of 26 µl per rat. This dose was not tolerated by any other route. The thermodynamic effects following rapid parenteral administration of the test material may be concluded to have occurred during inhalation. In any one single inhalation, 0.026 µl would be in the lungs, which may be below that necessary to produce trauma at a tissue surface. These data are considered further indications of the lack of acute systemic effects of the test compound.

TABLE III

FINDINGS IN RATS EXPOSED TO 200 ppm IN AIR FOR 10 MINUTES

Rat No.	Sacrifice	Serum		Tissue*		Blood	Fluo-	Protein	Electro-
		GOT	GPT	GOT	GPT	Glucose	ride	Plasma	pherogram Tissue
	(hours post-exposure)	units/ml		units/100g		mg per 100ml			
1	0	26	26	0	0	41	0**	N***	AN****
2	0	26	13	0	0	42	0	N	AN
3	0	42	14	0	10	44	0	N	AN
4	16	30	31	2160	2060	46	0	N	AN
5	died during exposure			-	-	-	-	-	-
6	16	16	16	4320	860	46	0	N	AN
7	16	17	20	4720	2120	44	0	N	AN
8	24	16	10	2511	1641	48	0	N	AN
9	24	21	16	3712	1721	44	0	N	AN
10	24	40	21	1916	870	47	0	N	AN

*Samples of right lung, upper lobe taken from each rat.

**0 indicates less than 2 mg per 100 ml.

***N indicates no abnormal protein bands in pattern (macroglobulins between the β and γ).

****AN indicates abnormal protein bands in pattern (macroglobulins between the β and γ).

A more detailed study of such effects was made by exposures of 40 rats to 100 ppm in air, 15 minutes per day for 6 consecutive days. At this level, the postulated daily exposure would be 12 µl of Compound A per animal. The data in tables IVa and IVb summarize the findings in the three animals per group sacrificed immediately and 16 hours after each exposure. They parallel those from rats exposed to 400 ppm in air, with the notable exception of prolonged hexobarbital sleeping time after the second exposure. At the same time, the nitrogen balance became negative. Thus, some systemic effects were noted following subacute administration of Compound A by inhalation. Although the significance of the negative nitrogen balance under these conditions is not known at this time, it may have been due simply to reduced food intake. Anorexia is often seen early in inhalation exposure studies. The animals showed marked weight loss, and roughness of fur coat at the time of sacrifice late in the study when some changes in indocyanine green excretion became evident. However, no specific indication of hepatotoxicity was seen. This was demonstrated by the inability to produce alterations in any of the coagulation parameters, i.e., fibrinogen content or prothrombin, coagulation, clotting, thromboplastin generation and partial thromboplastin times, either on exposure of six rats to 400 ppm for 10 minutes (table V) or on exposure to 100 ppm for 15 minutes for 6 days (table VI). During the latter, i.e., subacute exposure to 100 ppm for 15 minutes per day for 6 days, respiratory enzyme activity (glutamic oxaloacetic transaminase) was absent in the lungs, but all other criteria of physiological status were normal.

TABLE IVa

WEIGHT, FOOD CONSUMPTION, AND URINE NITROGEN OUTPUT OF RATS EXPOSED TO 100 PPM IN AIR, 15 MINUTES PER DAY FOR 6 DAYS

Rat No.*	Sacrifice (hours post-exposure)	Food Consumption (grams)								Urine Nitrogen Output ($g \times 10^2$)							Body Weight (grams)	
		0**	1	2	3	4	5	6	7	0**	1	2	3	4	5	6	Initial	Final
		days								days								
C-1	0	15	S***							24	S						300	300
C-2	0	14	S							23	S						301	301
C-3	0	15	S							24	S						302	302
1	0	14	S							23	S						303	303
2	0	16	S							23	S						304	304
4	0	16	S							21	S						306	306
5	16	14	S							23	S						307	307
6	16	17	S							24	S						308	308
7	16	12	S							20	S						309	309
8	0	17	17	S						21	19	S					310	309
9	0	16	10	S						26	31	S					301	300
10	0	14	0	S						21	32	S					302	301
11	16	16	10	S						26	31	S					303	300
12	16	15	12	S						21	36	S					304	305
13	16	21	13	S						22	19	S					305	305
14	0	16	10	0	S					23	36	32	S				306	300
15	0	16	11	9	S					25	30	30	S				307	300
16	0	14	12	6	S					26	28	36	S				309	307
18	16	16	12	12	S					21	21	19	S				310	310
19	16	15	14	8	S					24	26	26	S				301	300
20	16	15	20	10	S					30	31	28	S				302	302
21	0	15	10	6	2	S				26	30	31	16	S			303	295
22	0	16	8	6	2	S				30	29	16	14	S			304	300
24	0	16	6	5	6	S				26	28	27	16	S			316	300
25	16	16	10	10	2	S				26	31	30	17	S			317	299
26	16	16	11	4	8	S				17	34	31	12	S			318	310
28	16	17	17	17	2	S				20	30	36	18	S			310	296
29	0	17	11	6	4	2	S			26	21	17	21	S			317	300
30	0	12	9	6	2	2	S			21	26	29	16	16	S		310	300
31	0	17	10	0	4	4	S			26	27	28	17	4	S		319	310
32	16	16	11	9	3	2	S			23	27	19	20	10	S		319	299
33	16	15	12	12	4	0	S			23	29	18	16	9	S		320	285
34	16	15	15	14	4	0	S			31	37	26	14	16	S		321	285

All times shown designate period associated with that day's exposure.

*Animals C-1, C-2, and C-3 were chosen at random from the colonies of these Laboratories for use as controls.

**0 refers to the day prior to the first exposure.

***S = sacrifice

TABLE IVb

BIOCHEMICAL AND PHARMACOLOGICAL FINDINGS IN BLOOD AND TISSUES FROM RATS EXPOSED TO 100 PPM IN AIR, 15 MINUTES PER DAY FOR 6 DAYS

Rat No.*	Sacrifice (hours post exposure)	Glutamic Oxaloacetic Transaminase			Blood Fluoride	Indocyanine Green Retention	Electropherograms**		Hexobarbital Sleeping Time***
		Serum	Lung	Liver			Hemoglobin	Plasma	
		units/ml	units/100g		mg/100ml	per cent			min
C-1	0	37	3250	6130	0	0	N	N	40
C-2	0	36	1760	5620	0	0	N	N	46
C-3	0	16	860	9210	0	1	AN	N	37
1	0	26	0	6120	0	1	AN	N	40
2	0	16	0	5610	0	0	AN	N	41
4	0	31	0	3716	0	0	AN	N	42
5	16	26	2160	5360	0	1	AN	N	36
6	16	21	1050	6020	0	0	AN	N	40
7	16	17	820	6160	0	0	AN	N	46
8	0	15	0	5670	0	0	AN	N	36
9	0	16	0	9160	0	1	AN	N	39
10	0	31	0	2360	0	0	AN	N	42
11	16	16	1320	4720	0	0	AN	N	42
12	16	23	2160	8240	0	1	AN	N	39
13	16	16	2050	6700	0	6	AN	N	40
14	0	19	0	7210	0	0	AN	N	51
15	0	23	0	7280	0	0	AN	N	56
16	0	24	0	6720	0	1	AN	N	57
18	16	24	960	8610	0	0	AN	N	54
19	16	36	1420	7120	0	1	AN	N	53
20	16	37	2160	6010	0	0	AN	N	60
21	0	28	0	4160	0	0	AN	N	60
22	0	29	0	7020	0	2	AN	N	59
24	0	16	0	8060	0	0	AN	N	62
25	16	31	2160	6120	0	0	AN	N	75
26	16	26	1960	8160	0	0	AN	N	61
28	16	17	1120	6420	0	1	AN	N	64
29	0	20	0	6730	0	5	AN	N	76
30	0	20	0	9020	0	0	AN	N	80
31	0	19	0	8070	0	0	AN	N	61
32	16	36	2020	7160	0	1	AN	N	76
33	16	18	2720	6670	0	2	AN	N	77
34	16	27	1320	5340	0	2	AN	N	71

All times shown designate period associated with that day's exposure.

*Animals C-1, C-2, and C-3 were chosen at random from colonies of these Laboratories for use as controls.

**N = normal pattern; AN = abnormal pattern (macroglobulins between the β and γ fractions).

***After injection of 100 mg sodium hexobarbital (Evipal Sodium, Winthrop-Stearns) per kg body weight.

TABLE V

EFFECTS OF EXPOSURE TO 400 PPM FOR 10 MINUTES ON BLOOD COAGULATION IN THE RAT

Rat No.*	Prothrombin Time	Coagulation Time	Clotting Time	Thromboplastin Generation Time	Partial Thromboplastin Time	Fibrinogen
	seconds					g/100 ml
C-1	13.1	95	205	19.4	101	0.48
C-2	14.1	87	235	18.2	85	0.42
2	12.9	96	187	19.4	98	0.47
3	13.6	81	216	20.6	96	0.37
4	12.9	72	210	17.6	82	0.51
6	13.7	82	251	19.8	93	0.41
8	15.0	84	210	17.7	81	0.42
9	14.6	84	216	18.2	90	0.46
10	14.1	84	256	18.1	86	0.47

*C-1 and C-2 were rats selected at random from the stock colonies of these Laboratories for control purposes.

TABLE VI

EFFECTS OF EXPOSURE TO 100 PPM FOR 15 MINUTES FOR 6 DAYS IN THE RAT

Day of Sacrifice	No.of Rats	Prothrombin Time*	Glutamic Oxaloacetic Transaminase Activity*		Indocyanine Green Retention**	Hexobarbital Sleeping Time**
			Lung	Liver		
		sec	units/100 g		percent	minutes
1	3	16.1	0	6320	0,0,0	42,50,43
2	3	15.9	26	4212	0,0,1	38,42,41
3	3	16.1	14	9061	1,0,1	39,60,41
4	2	15.5	0	7600	4,2	60,76
5	2	16.1	36	6020	6,0	60,61
6	2	15.0	112	5136	4,6	82,61
7	2	14.9	0	5270	6,5	81,60
8	2	15.5	26	8160	8,4	46,87

*On pooled samples from all rats in group.

**Individually in each rat of the group.

Failure to detect increases in serum or tissue fluoride levels may have been due to the fact that this particular inorganic anion is sequestered in osseous tissue very rapidly. Since bone fluoride levels were not investigated in this study, no statement can be made as to the total body fluoride levels.

Intravenous Infusion Studies

Earlier observations had indicated that parenteral administration of Compound A caused death due to hemorrhage and trauma, both of which were apparently sequelae to massive evolution of the energy of hydrolysis and/or decomposition. A second parenteral study was carried out with the test material introduced intravenously at a very slow rate. In these experiments, 100, 20, 10, and 1 µl Compound A were infused.

The results are presented in tables VII, VIII, IX, and X, respectively.

Introduction of a total of 100 µl during a 15-minute period produced vascular trauma despite the fact that the injection rate was 1 µl per second in the first rabbit used. The total amount injected at the time of death was about 60 µl. Clonic convulsive activity with anoxia and death were noted. At necropsy, dilation of major abdominal vessels and destruction of the ear vein, the site of injection, were observed. The thoracic cavity was filled with blood and the lungs were completely hemorrhagic. Enzyme activity was normal in all tissues but the abnormal "s"-like pattern was seen in the hemoglobin electropherogram. Treatment of the second rabbit at a rate of 1 µl each 5 seconds caused death within 5 minutes, after approximately 60 µl were administered. The biochemical and necropsy findings were almost identical to those described in the first rabbit. Reduction of the rate of infusion to 1 µl every 20 seconds caused a similar death in 10 minutes, after 30 µl were introduced. The necropsy findings and biochemical data were similar to those of the other rabbits in which more than 25 µl were administered. Fluoride levels in the blood, brain, heart, liver, and lung could not be detected.

TABLE VII

RABBITS TREATED WITH 100 µl BY INTRAVENOUS INFUSION

Rabbit	Survival		Electropherogram	GOT			
No.	Rate	Minute	Hemoglobin[1]	Brain	Heart	Liver	Lung
	µl/sec			units/100 g			
1	1	1	"s" present	560	1921	5126	971
2	1/5	5	"s" present	652	2640	4670	1011
3	1/20	10	"s" present	830	926	7260	516

[1] "s" = abnormal pattern

TABLE VIII

FINDINGS IN RABBITS RECEIVING 20 µl BY INTRAVENOUS INFUSION

Rabbit No.	Time after Injection	SGOT	Pro-thrombin Time	Electro-pherogram Hemoglobin*	Fluo-ride	Glucose	Urea Nitrogen
	minute	units/ml	sec			mg/100 ml	
1	pretest	22	9.4	N	0	46	13
	0**	12	9.4	N	0	48	13
	15	26	9.1	"s"	0	46	13
	30	40	9.4	"s"	0	56	13
	60	28	9.7	"s"	0	60	14
	360	20	8.6	"s"	0	46	13
2	pretest	20	7.1	N	0		
	0	18	7.6	N	0	61	14
	15	6	7.6	N	0	70	11
	30	10	7.5	N	0	76	16
	60	12	6.7	N	0	62	11
	360	18	7.9	N	0	60	17
3	pretest	36	8.6	N	0	44	11
	0	12	8.6	N	0	48	11
	15	10	8.9	"s"	0	46	11
	30	36	10.1	N	0	44	11
	60	32	10.2	N	0	48	11
	360	34	9.5	N	0	50	11

*"s" = abnormal pattern; N = no abnormal bands in pattern.

**0 = samples taken immediately upon cessation of infusion.

TABLE IX

FINDINGS IN RABBITS RECEIVING 10 μl BY INTRAVENOUS INFUSION

Rabbit No.	Survival	Time after Administration	Serum		Electropherogram		Blood		
			GOT	GPT	Plasma Protein	Hemoglobin*	Fluoride	Glucose	Urea Nitrogen
	hrs		units/ml				mg/100 ml		
1	11	pretrial	46	21	N	N	0	51	14
		0**	0	0	N	"s"	0	56	14
		15	0	0	N	"s"	0	60	14
		30	46	0	N	"s"	0	48	13
		60	16	20	N	"s"	0	49	16
		360	31	20	N	N	0	55	14
		660	36	26	N	N	0	60	15
2	13	pretrial	23	16	N	N	0	55	16
		0	0	0	N	"s"	0	61	17
		15	0	0	N	"s"	0	62	16
		30	23	10	N	"s"	0	76	14
		60	31	20	N	N	0	55	15
		360	16	10	N	N	0	59	20
		720	16	20	N	N	0	68	15
3	18	pretrial	30	30	N	N	0	60	11
		0	0	0	N	"s"	0	60	12
		15	0	21	N	"s"	0	65	11
		30	0	26	N	"s"	0	61	14
		60	0	30	N	"s"	0	62	11
		360	21	16	N	"s"	0	60	9
		720	16	20	N	N	0	59	14

*"s" = abnormal pattern; N = no abnormal bands in pattern.

**0 = samples taken immediately upon cessation of infusion.

TABLE X

FINDINGS IN RABBITS RECEIVING 1 μl BY INTRAVENOUS INFUSION

Rabbit No.	Time after Administration	Prothrombin Time	SGOT	Electropherogram	Blood		
				Hemoglobin*	Urea Nitrogen	Glucose	Fluoride
		sec	units/ml		mg/100 ml		
1	pretrial	11.0	32	N	11	44	0
	0**	10.5	32	N	12	46	0
	15	11.8	26	N	14	48	0
	30	10.8	28	N	11	60	0
	45	11.1	32	N	16	52	0
	60	10.9	29	N	14	44	0
	360	10.6	33	N	12	44	0
	1080	10.5	40	N	15	48	0
	1440						
2	pretrial	7.6	20	N	11	44	0
	0	7.8	20	N	16	44	0
	15	8.1	20	N	20	44	0
	30	7.9	22	N	16	44	0
	45	7.6	20	N	16	60	0
	60	9.5	26	N	11	66	0
	360	9.1	31	N	16	40	0
	1080	8.6	18	N	8	56	0
	1440	7.6	39	N	8	58	0
3	pretrial	8.1	18	N	14	48	0
	0	8.1	16	N	14	46	0
	15	8.1	21	N	14	46	0
	30	7.5	36	N	14	45	0
	45	6.9	26	N	21	46	0
	60	6,9	18	N	20	49	0
	360	7.4	21	N	12	46	0
	1080	7.5	16	N	16	44	0
	1440	6.9	29	N	14		0

*N = no abnormal bands in pattern

**0 = Samples taken immediately upon cessation of infusion.

With the administration of 10 or 20 μl of the material, respiratory enzyme activity in the serum fell to undetectable levels. Whether this was due to inhibition by the fluoride ion itself or to some physical reaction denaturing the protein, is not clear from these data although the fluoride concentration never rose significantly above 20 μgm per ml. As before, the most significant finding in all the studies, in which the level of administration exceeded 10 μl per animal, was the unusual band in the hemoglobin electropherograms. This was seen for 60 minutes postinjection. Whereas this band had a migration pattern similar to that of human hemoglobin "s", the abnormal protein noted cannot be identical to the human protein since the latter differs from the normal in amino acid content. Nevertheless, a sample of hemoglobin "s" was secured from the Hyland Laboratories (Los Angeles, California) and used as a control. The migration characteristics of the variant in the treated rabbit serum and of the hemoglobin "s" were similar. Since we cannot assume that a de novo synthesis of the "s" form occurred at or near the site of injection, these data suggest that the decomposition or hydrolysis of Compound A was accompanied by a conformation change in normal rabbit hemoglobin. Sections of these abnormal hemoglobins were recovered from the electropherograms and tested for fluoride content, but none could be detected.

The rabbits receiving 20 μl per animal died in convulsions 6, 6.4, and 8 hours postinjection while those receiving 10 μl per rabbit at the rate of 0.5 μl per minute died after 11, 13, and 18 hours, showing the same behavioral signs noted at the higher doses. The findings of all animals at necropsy included massive myocardial and/or pulmonary infarctions probably associated with thromboembolic phenomena. Intravenous administration of 10 μl or more of Compound A may be concluded to have caused some localized denaturation of protein with alterations in respiratory enzyme and hemoglobin patterns. The denaturant effects probably affected the colloidal state of blood proteins producing either emboli or nuclei for thrombus formation, leading to massive clotting, shock, and death.

All rabbits receiving 1 μl of Compound A by intravenous infusion survived the 24-hour observation period (table XI). No abnormalities were noted in any of the chemical criteria, blood, or tissue analyses. Necropsy of these animals sacrificed 24 hours after injection revealed some signs of localized tissue destruction at the site of injection, but no changes in the morphology of the cardiovascular, hepatic, or brain tissues. Apparently this dose when administered very slowly did not cause sufficient physical changes to affect either organ morphology or serum enzyme function.

TABLE XI

FINDINGS IN RABBITS RECEIVING DAILY INTRAVENOUS INFUSIONS OF 1 μl

Rabbit No.	Day of Sacrifice	Day of Determination	SGOT	Blood Fluoride	Prothrombin Time	Electropherogram Hemoglobin*
			units/ml	mg/100 ml	sec	
1	5	pretrial	41	0	9.4	N
		0**	36	0	8.5	N
		5	20	0	8.6	N
2	5	pretrial	26	0	7.9	N
		0	28	0	8.5	N
		5	16	0	9.6	N
3	5	pretrial	16	0	10.1	N
		0	10	0	7.5	N
		5	26	0	7.9	N
4	20	pretrial	38	0	6.6	N
		0	26	0	7.5	N
		5	34	0	9.6	N
		10	26	0	7.9	N
		20	18	0	8.8	N
5	20	0	16	0	8.8	N
		5	18	0	8.8	N
		10	40	0	7.6	N
		20	36	0	8.4	N
6	20	pretrial	40	0	9.6	N
		0	26	0	7.9	N
		5	46	0	6.9	N
		10	38	0	8.1	N
		20	18	0	9.1	N

*N = no abnormal bands in pattern.

**0 = samples taken immediately upon cessation of infusion.

SUMMARY

The effects of acute and subacute administration of Compound A have been studied in rats, rabbits, cats, and guinea pigs using the intragastric, intraperitoneal, intravenous, subcutaneous, and inhalation routes. Ten, 25, 50, or 100 µl of Compound A per animal caused immediate death in all four species when given parenterally or orally.

Dermal administration of 10 µl per animal caused massive irritation with destruction of the skin and subsequent trauma. Intravenous infusion of 20 or 10 µl per rabbit over 15 minutes caused the death of groups of rabbits 8 and 12 hours later, respectively, with signs of massive hemorrhage and infarcts in the heart and lung, whereas slow infusion of 1 µl of Compound A per animal was tolerated as a single dose or when given for as many as 20-consecutive daily doses.

Graded effects were seen during inhalation studies, with no rats surviving more than 10 minutes of exposure to 400 ppm of the test material in air. Thirty percent of rats exposed to 200 ppm for 10 minutes survived for 24 hours while almost all rats survived exposure to 100 ppm for 15 minutes daily up to 5 days. In the latter group, all rats had lost weight at end of the exposure period.

The observations made indicate that rapid administration of 10 µl or more of Compound A per animal was followed by the rapid evolution of energy due either to some form of autolytic decomposition or to reaction of this labile material with body fluids. No adverse effects on clotting were found with administration of the test material. Thus, the most significant findings seen were inhibition or absence of enzyme activity and alterations in protein structure immediately after topical parenteral, or dermal administration at or near the site of administration.

Such changes may be attributable to the exothermic reaction of Compound A with physiological substances or to the fluoride-ion alone.

At the tolerated levels administered as single or multiple subacute doses, Compound A probably exerted no pharmacological action. Where effects were noted, these resulted from the energy release following administration.

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13. ABSTRACT

Studies are described in which parenteral, oral, topical, and inhalation administration of Compound A (ClF_5) have been made to rats, cats, guinea pigs, and rabbits. Administration of microliter quantities of the material caused traumatic explosions and death in most animals. The notable findings in survivors reflected protein alterations which were considered to be sequelae to massive evolution of the energy of hydrolysis and/or decomposition. No pharmacological or biochemical activity of the material could be demonstrated at tolerated doses.

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